PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	05.02.99
Applicant's or agent's file reference: IPD/P1174/WOD	**REPLY DUE** within **3 month(s)** from the above date of mailing

International application no.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB98/01026	07/04/1998	11/04/1997

International Patent Classification (IPC) or both national classification and IPC
C12N9/02

Applicant
THE SECRETARY OF STATE FOR DEFENCE et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This report contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☒	Certain defects in the international application
VIII	☒	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and / or arguments, see Rule 66.4bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 11/08/1999

Name and mailing address of the international preliminary examining authority

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I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*):

Description, pages:

1-15	as originally filed				

Claims, No.:

11-35	as originally filed				
1-10	as received on	06/11/1998	with letter of	29/10/1998	

Drawings, sheets:

1/9-9/9	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application,

☒ claims Nos. 6, 13, 17,

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☒ the claims, or said claims Nos. 6, 13, 17 are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-5, 14-16, 18, 22-24, 25-27, 30-31
Inventive step (IS)	Claims	
Industrial applicability (IA)	Claims	

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Present claims 6, 13 and 17 do not comply with the requirements of Article 6 PCT for the following reasons:

1.1. Dependent claim 6 relates to a recombinant luciferase having a V_m for ATP at least 5-100% of wild type. Although it is stated on page 5, lines 1-5 of the description, that the activity for ATP of the mutant should be 5-100% of the wild-type, the V_m of the mutant luciferases has not been assessed in any of the examples. Said claim thus appears to lack support by the description.

1.2. Claim 13 relates to a fusion protein comprising a mutant luciferase. Apart from a brief mention in the description (page 7, lines 6 ff), no disclosure is made regarding the features of said fusion protein. Especially in light of prior art document D1 (see below), it would appear that a fusion protein containing additional amino acids, like the recombinant wild-type luciferase of D1, may have decreased stability due to conformational changes. Accordingly, the fusion protein of claim 13 lacks support from the description.

1.3. Claim 17 relates to a vector encoding a mutant luciferase under the control of a tissue or organ specific promoter. Such specific promoters are not supported by the description and do not comply with the provisions of Article 6 PCT.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2.1. Reference is made to the following documents:

D1: E.I. Dementieva et al., BIOCHEMISTRY, vol. 61, no. 1, 1996, pages 115-119
D2: E.I. Dementieva et al., BIOCHEMISTRY, vol. 61, no. 7, 1996, pages 915-920

2.2. D1 discloses a recombinant luciferase with catalytic and spectral properties similar to the native one, albeit with decreased stability. Variants with point mutations however, showed a 4-8-fold increase in K_m values for ATP, ranging from 600µM-1.2mM (Table 1), and **similar stability** to the native luciferase (Fig. 1). One mutant variant (Cys-393→Ala), appeared to be **more stable** than the native luciferase (Fig. 1, open square).

D2 discloses the monitoring of ATP concentration in intact cells expressing recombinant luciferase. The K_m for ATP of luciferase in aqueous solution is 0.15mM. The K_m for the recombinant luciferase inside the cells appeared to be 4.6mM (page 919, right hand column).

2.3. The gist of the present application is that substitution of the amino acid corresponding to amino acid 245 in Photinus pyralis, for a hydrophobic amino acid (Ala, Asn or Gln), results in a mutant enzyme with a K_m higher than the wild-type, and a thermostability at least as high as the wild-type enzyme. It is noted that throughout the application the term "stability" is used instead of the more specific term "thermostability", which leads to unclarity with respect to the scope of protection of the claimed subject-matter (see item 8.1).
Luciferases with point mutations resulting in enzymes with similar properties (Increased K_m **and** thermostability) were known from the prior art (see D1). Thus, all claims relating to recombinant mutant luciferases, as defined by claims 1-5, recombinant vectors and host cells, processes, methods and kits, lack novelty in light of prior art document D1.
Recombinant luciferases with point mutations at amino acid 245 are not disclosed in the prior art, nor has the importance of this position been anticipated. Thus, the recombinant luciferases of claims 7-10 as well as the following vector, host cell and method claims **in as far as they refer to these claims only**, appear to be both novel and inventive under the terms of articles 33(2) and (3) PCT. For the drafting of an amended set of claims that would meet the criteria of Rule 6 PCT, the applicant is referred to items 4.1-4.3 (see below).

Re Item VII
Certain defects in the international application

3.1. To meet the requirements of Rule 5(1)(a)(ii) PCT, prior art document D1 should be identified in the description and the relevant background art disclosed therein should be briefly discussed.

3.2. Claim 19 appears to be redundant in light of preceding claim 18 and should thus be deleted in compliance with Rule 6(1)(a) PCT.

Re Item VIII
Certain observations on the international application

4.1. The term "stability" used in claim 1 should be replaced with the more specific term "thermostability" for he purpose of clarity and conciseness under the terms of Article 6 PCT.

4.2. Claims 1, 7, 12 and 21 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. Said claims attempt to define the subject-matter in terms of the result to be achieved which merely amounts to a statement of the underlying problem. The technical features necessary for achieving this result should be added (see item 2.3).

4.3. The terms "increased", "double", "five times higher", "at least as high" and "5-100%", used throughout the claims are relative terms that, unless used in relation to a standard value, are meaningless and unclear. Reference values, i.e. the

corresponding values for recombinant wild type luciferase from Tables 1 and 2, should be included in the claims to restore clarity, in compliance with Article 6 PCT.

4.4. The subject-matter of claim 8 appears to be an essential feature of the disclosed invention and should thus be incorporated in any independent claim relating to the mutant luciferase of the invention.